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Extended Summaries Resistance '97

The following are extended summaries based on presentations at the international conference 'Resistance '97—Integrated Approach to Combating Resistance' organised by the Institute of Arable Crops Research in collaboration with the SCI Pesticides Group and the British Crop Protection Council and held at IACR-Harpenden, Herts., UK on 14–16 April 1997. They are entirely the responsibility of the authors and do not necessarily reflect the views of the Editorial Board of Pesticide Science.

Acaricide Resistance Management of Leprosis Mite (*Brevipalpus phoenicis*) in Brazilian Citrus

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Introduction

The Brazilian citrus industry is responsible for more than 30% of world citrus production.[1] In order to compete in a global market, new production technologies are constantly reviewed and adopted by citrus producers. At the moment, among major problems that affect citrus production in Brazil, the phytophagous mite *Brevipalpus phoenicis* (Geijskes) is one of the most serious pests because it transmits citrus leprosis virus.[2–4] Symptoms of the leprosis disease appear on fruits, leaves and stems and can lead to premature leaf and fruit drop, or even the death of the plant. For its control, great progress in the area of integrated pest management (IPM) has been achieved by Brazilian scientists. Studies on the bioecology of leprosis mite,[5–7] sampling procedures,[8] cultural practices,[6,9] pesticide selectivity,[10,11] and biological control[11–13] have all contributed to improve leprosis mite management in citrus groves. However, we still rely on the use of chemicals for effective control of this pest.

Approximately US$80 million are spent per year on acaricides in Brazilian citrus groves, which represents 21% of the citrus production cost.[14] Acaricide use has increased due to the occurrence of another important acarine pest, the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead). Both *B. phoenicis* and *P. oleivora* occur throughout the year on citrus trees. However, the critical period of mite infestation differs for each species. *P. oleivora* is a major problem during the summer and fall seasons when the temperature and relative humidity are very high. On the other hand, population densities of *B. phoenicis* start increasing when the relative humidity is lower, i.e. during the winter and spring seasons under Brazilian conditions.[6] Hence acaricides are used throughout the citrus growing season with an average of two to four applications per year.

Failure to control *B. phoenicis* with conventional acaricides has been reported frequently by citrus growers and advisors. Interestingly, control problems with this pest have also been detected for some new molecules that are coming to the market. Because of constant selection pressure with acaricides, the evolution of resistance in *B. phoenicis* could be one of the major factors affecting the efficacy of some products. Despite intensive use of acaricides on citrus in Brazil, there is a lack of research on the detection and monitoring of pest resistance to pesticides. This summary reports work to implement acaricide resistance management for *B. phoenicis* in Brazilian citrus.

Factors affecting the evolution of resistance in Brevipalpus phoenicis

Factors that may promote the development of resistance in *B. phoenicis* include its mode of reproduction, predominantly by thelytoky, and the karyotype of only two heterologous chromosomes.[15] From an evolutionary perspective, thelytoky is often thought to be an evolutionary 'dead end' because it increases homozygosity and mutational load, and limits or inhibits genetic recombination. However, the holokinetic chromosome structure and inverted meiosis of *B. phoenicis* have been proposed as contributing to its evolutionary success.[16] Under thelytoky, females develop from unfertilised eggs (identical to maternal genome). If there is genetic variation for resistance in a population of *B. phoenicis*, selection pressure with an acaricide should rapidly increase the proportion of resistant genotypes. In theory, the probability of detecting individuals with two or more

resistance mechanisms (multiple resistance) is high because *B. phoenicis* females are haploid with only two chromosomes.[15,16] This needs to be appreciated when formulating tactics such as rotations or mixtures of acaricides for managing resistance.

However, there are also factors that favor the retention of susceptibility to acaricides. For example, *B. phoenicis* is a very polyphagous species. Over 30 different host plants (including cultivated plants and weeds) have been identified in the State of São Paulo (the major citrus production area in Brazil).[7] These hosts could serve as refuges for susceptible mites. However, the role of refuges should be investigated in more detail to establish the relative influences of thelytoky and sexual reproduction in restricting or promoting genetic mixing with susceptible migrants. Many natural control agents have been identified for controlling *B. phoenicis*, such as Phytoseiid predators,[11–13] which could contribute to reducing the frequency of resistant mites. Most importantly, IPM programs are well-established in the Brazilian citrus industry.[17,18] However, recognising the threat of resistance to pesticides is critical for all IPM programs involving the use of chemicals.[19,20] The next essential step for improving the control of citrus pests in Brazil is to incorporate pesticide resistance management strategies into IPM programs in order to define a more rational use of chemicals, particularly for acaricides which represent a significant fraction of the total cost of citrus production.

Developing acaricide resistance management of Brevipalpus phoenicis *in Brazilian citrus*

Studies on the detection and monitoring of *B. phoenicis* resistance to acaricides in Brazillian citrus were started by the Rohm and Haas Chemical Company in 1988 to investigate reports of control failures with the acaricide dicofol. These studies were coordinated by Rohm and Haas personnel and public sector scientists including T. J. Dennehy (Cornell University, Geneva, USA) and S. Gravena (UNESP—Universidade Estadual Paulista, Jaboticabal, SP, Brazil). From this preliminary work, a strategy of rotating different groups of acaricides was suggested to citrus growers.[21] However, many questions relating to the status of resistance of *B. phoenicis* to other acaricides, as well as the frequency with which acaricides should be used in a rotation strategy, remain to be resolved. To address these questions, a research project on acaricide resistance management for *B. phoenicis* in Brazil was started in 1996 at the Laboratory of Pesticide Resistance Management, Department of Entomology of University of São Paulo, Piracicaba, SP, Brazil.

Initially we defined bioassay procedures to evaluate the susceptibility of different *B. phoenicis* populations to the major groups of acaricides, i.e. organotins (fenbutatin oxide and cyhexatin), organochlorines (dicofol), sulfite esters (propargite) and carboxamides (hexythiazox), which represent approximately 44, 25, 13 and 10% of acaricides used for controlling *B. phoenicis*, respectively. Based on concentration-response lines for a susceptible reference population, we defined diagnostic concentrations of each acaricide for surveying spatial variability in the frequency of resistance in populations from commercial citrus groves. Selection experiments are being conducted in the laboratory in order to isolate and characterise individuals resistant to each compound. Using such resistant colonies, we are investigating cross-resistance or multiple resistance of acaricides registered for use in citrus. Finally, in order to understand the dynamics of resistance to each compound, temporal variability in the susceptibility of *B. phoenicis* to acaricides is being monitored in the field. This will serve to indicate how resistance frequencies change in response to the presence and absence of selecting agents. Any observed instability in resistance should be exploited through appropriate rotation of acaricides.[19,22]

This research project will provide important and practical information for understanding the severity and extent of resistance problems in *B. phoenicis*, and contribute to the construction of an acaricide resistance management program in Brazilian citrus. However, for effective implementation of this program, cooperation between academics, chemical companies, citrus growers and consultants will be essential. Because pesticide resistance issues are relatively new in Brazil, educational efforts will play an important role in conveying the importance of resistance management. Fortunately, agochemical companies are now exploring the possibility of establishing an Insecticide Resistance Action Committee (IRAC) in Brazil; if successful, this would be a significant step towards effective implementation of pesticide resistance management programs in Brazil.

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Reduced Rates of Herbicide Metabolism Confer Tri-allate Resistance in *Avena fatua**

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Extensive use of the pre-emergence thiocarbamate herbicide tri-allate [*S*-(2,3,3-trichloroallyl di-isopropylthiocarbamate] over the last 15 to 20 years has selected for resistant (R) wild oat (*Avena fatua* L.) populations in several areas of Montana and Canada.[1,2] Our laboratory's goal was to discover the biochemical mechanism of resistance in order to: (i) better understand the exact mechanism of tri-allate's herbicidal action, (ii) characterize the strategies used by weed plants to escape herbicidal selection pressure, and (iii) generate basic information that may be used to develop field-scale resistance management strategies. R wild oat seeds were collected in August 1993 from fields near Fairfield, Montana in which tri-allate had been used annually for 15 to 22 years[3] from plants surviving treatment the preceeding spring with $1{\cdot}1$ kg ha^{-1} tri-allate. The field collections were shown in greenhouse and Petri dish dose-response experiments to be 6- to 20-fold more tolerant to tri-allate than susceptible (S) lines.[1] One of the R collections was used to develop the inbred R line reported here, through two generations of recurrent selection under $1{\cdot}1$ kg ha^{-1} tri-allate in the greenhouse. R populations and the inbred line were shown to be resistant (8-fold) to the related thiocarbamate herbicide di-allate, as well as to the chemically unrelated post-emergence herbicide difenzoquat (60-fold).[1] S wild oat seeds were collected from field-grown populations of the non-dormant inbred line SH430.[4]

To compare tri-allate uptake and translocation patterns in R and S plants, four-day-old seedlings were treated with [1-^{14}C]tri-allate (1 μl; $1{\cdot}67 \times 10^3$ Bq; sp. act. $3{\cdot}42 \times 10^6$ Bq mg^{-1}) in methanol on the apex of the coleoptile and incubated at 22($\pm$2)°C.[1] After 24, 48, or 60 h, shoots were harvested, washed three times with ethanol + water (90 + 10 by volume; 50 μl) to remove unabsorbed tri-allate, and oxidized in a biological

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